Fabrication of Multi-layered Crosslinked Functional Electrospun Nanoscaffolds as Substrates for Cell Culture

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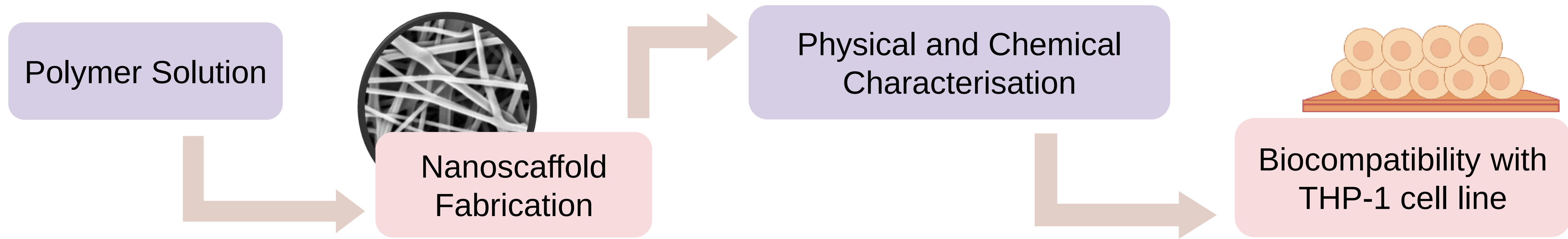
BACKGROUND

Electrospun nanofibres have been utilised in tissue engineering as nanoscaffolds due to their potential to mimic the structural and functional role of the extracellular matrix (ECM) architecture [1]. Electrospun nanofibres can be generated as single, bi- or multi-layered systems. Multi-layered Nanofibre can improve the porosity, pore diameter and mechanical properties than the single inner layer [2].

AIM

To fabricate multi-layered crosslinked functional electrospun nanoscaffolds with both synthetic and natural polymers that can serve as highlight versatile and biocompatible substrates for tissue engineering applications.



METHODS

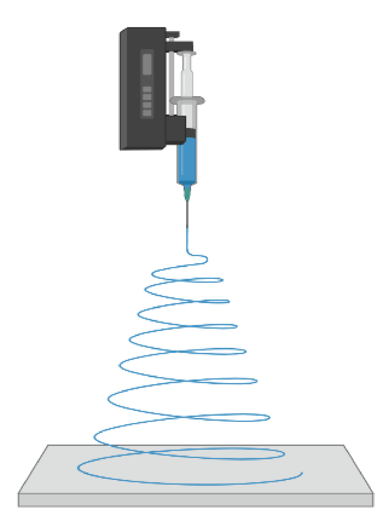
1. Solution Preparation

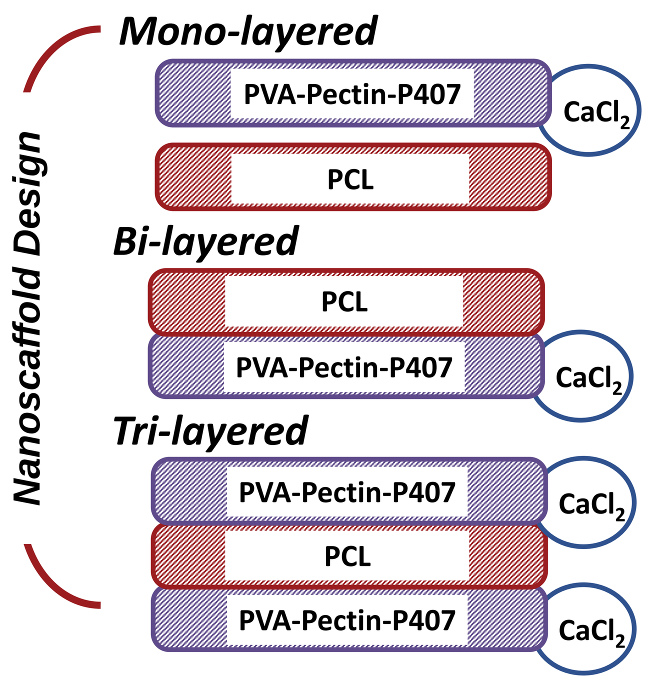
Natural Polymer :
- Pectin

Synthethic Polymer :
- Polyvinyl Alcohol (PVA)
- Polycaprolactone (PCL)
- Poloxamer (P407)

Crosslinking Agent :
- Calcium Chloride ($CaCl_2$)

2. Electrospinning





3. Characterisation

- **Scanning Electron Microscopy (SEM)**
  - ✓ Morphology
  - ✓ Fibre diameter
- **Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR)**
  - ✓ Hydrogen bonding
  - ✓ Confirmation of polymer incorporation
- **Moisture Content Analysis**
- **Porosity**
- **Cell Viability**
  - ✓ Alamar Blue method with THP-1 cell line

RESULTS

Scanning Electron Microscopy

Nanofibre Diameter

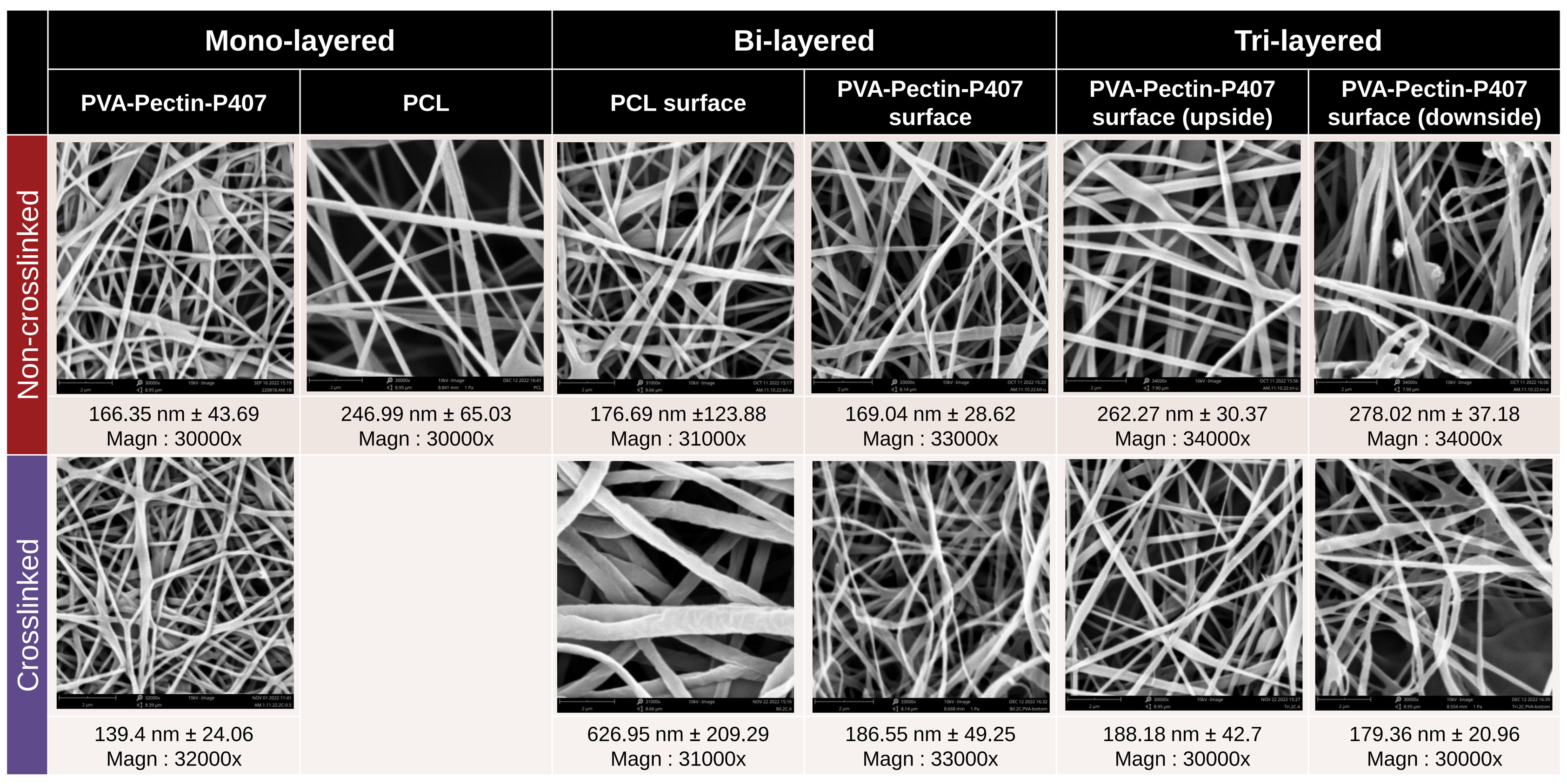


Figure 1. SEM images of electrospun nanoscaffolds generated and nanofibre diameter measurement

Nanofibrous Matt Thickness

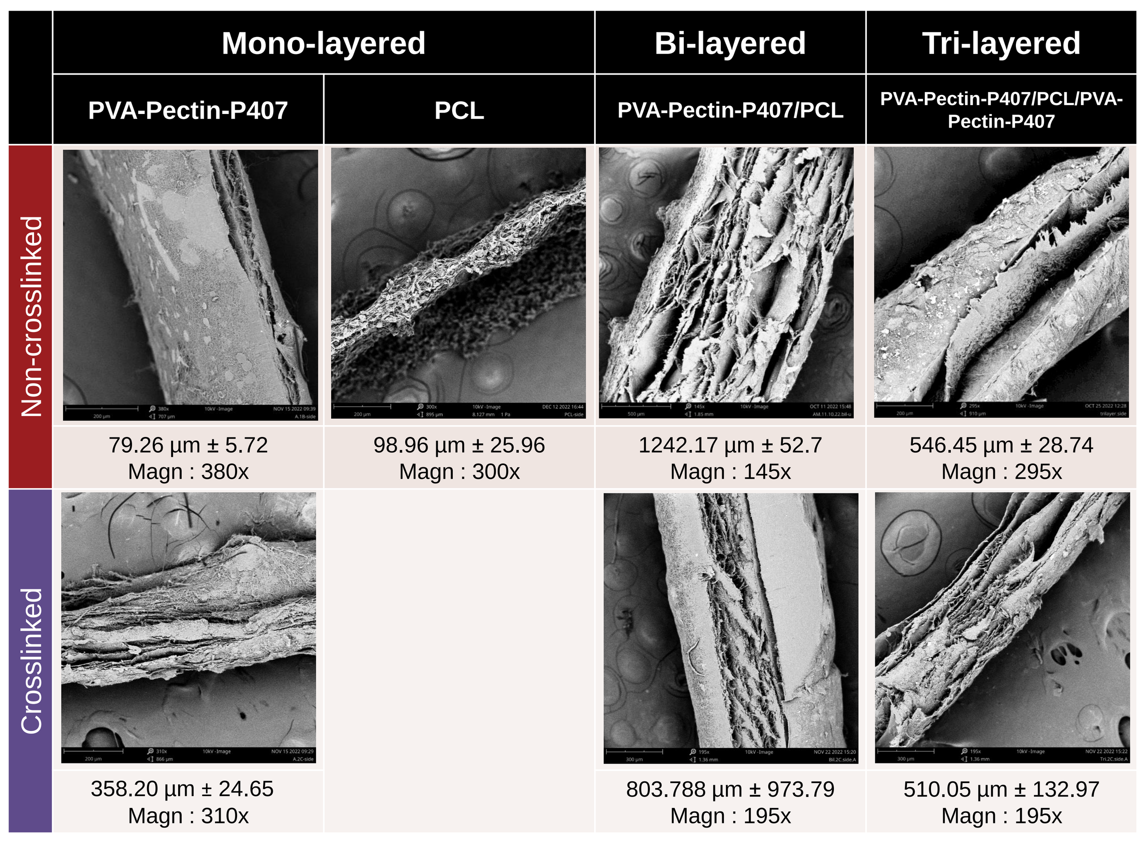


Figure 2. SEM images of electrospun nanoscaffolds and matt thickness measurement

All formula produced nanofibre with good morphology (without beads) with the fibre diameter within nanosize range. Bi-layered nanofiber create a thicker layer than the Tri-layered nanofiber, both for non-crosslinked and crosslinked formula. This indicates the effect of additional layer of PVA-Pectin-P407 to make the structure of nanofiber more compact thus reducing the thickness.

Moisture Content and Porosity

Table 1. Moisture Content and Porosity of Nanoscaffolds

	Layer-type	Sample Name	Moisture Content	Porosity
Non-Crosslinked	Mono-layered	PVA-Pectin-P407	0.98%	72.32%
		PCL	1.96%	94.43%
	Bi-layered	PVA-Pectin-P407/PCL	1.03%	86.71%
	Tri-layered	PVA-Pectin-P407/PCL/PVA-Pectin-P407	2.07%	84.59%
Crosslinked	Mono-layered	PVA-Pectin-P407	2.16%	83.63%
	Bi-layered	PVA-Pectin-P407/PCL	2.52%	85.31%
	Tri-layered	PVA-Pectin-P407/PCL/PVA-Pectin-P407	2.72%	84.62%

All formula produced nanofiber with the low moisture content (<3%) and high porosity (>70%). The addition of crosslinking agent and formation of multilayer increase the moisture content of nanofiber and enhance the nanofibrous matt porosity.

Attenuated Total Reflection – Fourier Transform Infrared Spectroscopy

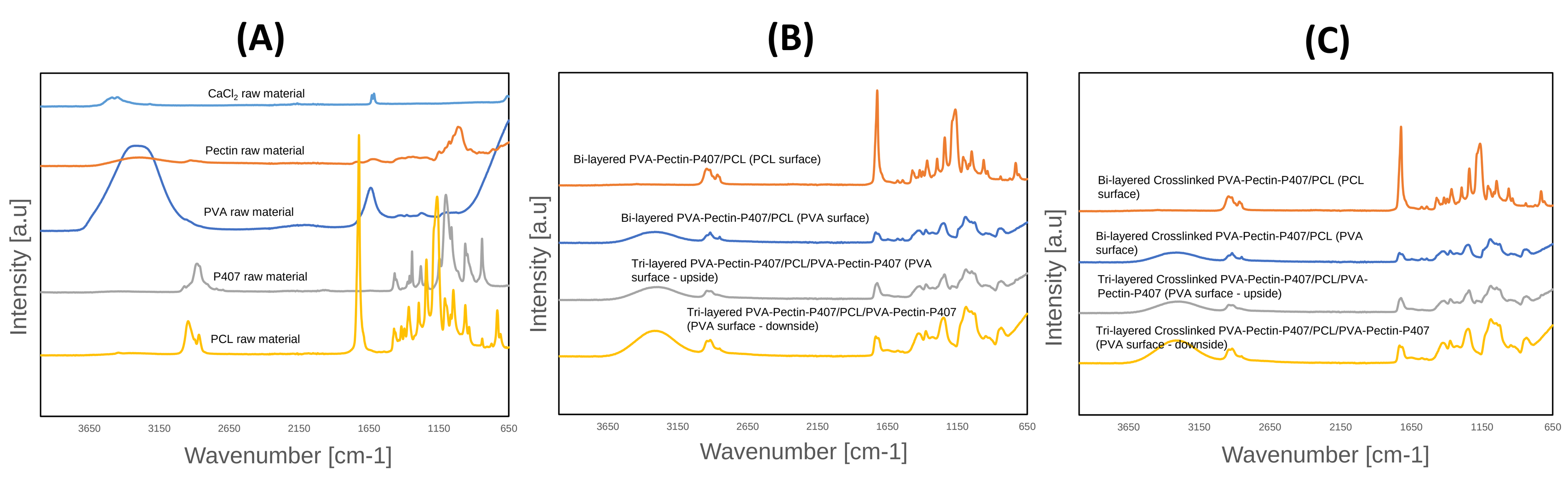


Figure 3. FTIR Spectra of (A) Nanoscaffold Raw Material; (B) Non-Crosslinked multilayer Nanoscaffolds; (C) Crosslinked Multilayer Nanoscaffolds

The FTIR Spectra clearly demonstrate occurrence of principal peak from raw material of both non-crosslinked and crosslinked formula of the multilayer nanofibre. The respective surface also shows the formation of layer which is in accordance with the design of each type of multilayer.

Cell Viability

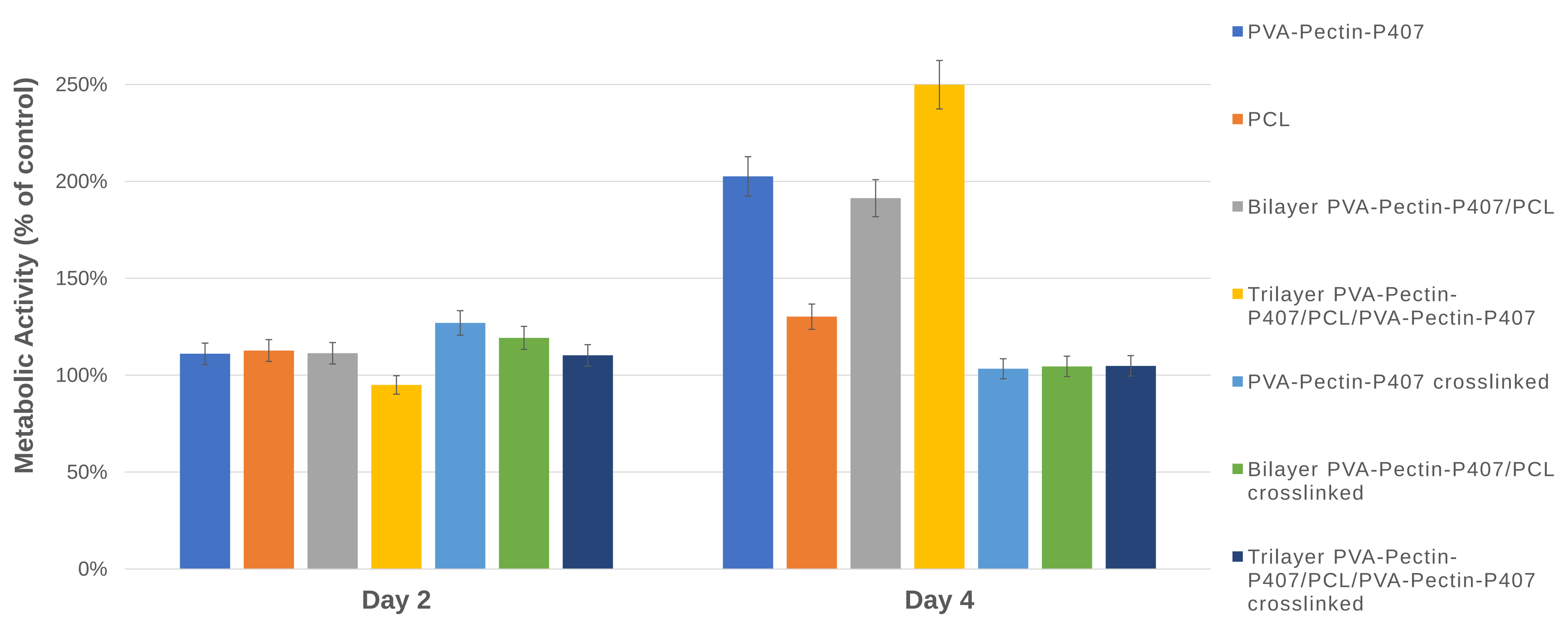


Figure 4. The mean fluorescence indicating the THP-1 cell viability when cultured on the samples

Cell proliferation is improved by the addition of nanofibre, with non-crosslinked formula giving a better improvement than crosslinked formula. PVA-Pectin-P407 induce the proliferation better than PCL.Trilayer non-crosslinked nanofibre produce the highest cell proliferation. Multi-layered non-crosslinked formula suggests an improved biocompatibility when compared to single layer and crosslinked formula.

CONCLUSION

In conclusion, the multi-layered and crosslinked electrospun nanoscaffold from PVA, pectin, P407, and PCL was effectively created with improved properties and suitability for the cells compared to the single-layered nanoscaffold. An implication of this research is the potential of developing the multi-layered design of nanoscaffolds that would offer superior biomimicry as synthetic ECM in the extensive field of tissue engineering.

REFERENCES

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Some illustrations created with BioRender.com

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